

A NEW SPECIES OF THE UNICORNFISH GENUS *NASO* (TELEOSTEI: ACANTHURIDAE) FROM TAIWAN, WITH COMMENTS ON ITS PHYLOGENETIC RELATIONSHIP

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ABSTRACT. – A unicornfish, *Naso tergus*, new species, was described based on 13 specimens collected by bottom gill-net and hook and line from Taiwan at the depth of 70–80 m. It shares with the *N. hexacanthus*/*N. caesius* clade, a morphologically similar group, by having VI dorsal-fin spines; an acute head without rostral protraction and tuberosity in adults; two bony plates on caudal peduncle, with a rounded blade-like spine on each plate, but differs from them in having a relatively slender body, its depth 2.8–2.9 in SL; no distinctive markings; and a relatively small adult body size. In addition to the distinctive morphological features, a phylogenetic reconstruction with all known *Naso* species indicates *N. tergus* is distinct from the *N. brevirostris*, *N. elegans* and *N. maculatus* sub-clades, which are of rapid evolutionary divergence from a common ancestor.

KEY WORDS. – Taxonomy, Acanthuridae, *Naso tergus*, new species, Phylogeny.

INTRODUCTION

Randall (2002) published a guide book on acanthurids of the world, recognizing 19 species of the genus *Naso*, 13 of which were recorded from Taiwan. In their recent comprehensive book, Chen et al. (2010) recorded 15 species of *Naso*, including two possible undescribed species, from the Kenting National Park in southern Taiwan. Among many field trips to fishing ports in northeastern Taiwan, the first author noticed some specimens of *Naso* lacking any distinctive markings on the body and fins and being different from any currently recognized species. Detailed comparisons with all known species showed these specimens are morphologically similar to *N. hexacanthus* (Bleeker, 1855) and *N. caesius* Randall & Bell, 1992, but different from them in proportional measurements and coloration.

Moreover, direct comparisons of the DNA sequences (ETS2, 16S, Cyt *b*) with all known species, as provided by Klanten et al. (2004), revealed that these specimens represent an undescribed species. A re-examination of the specimens

referred to as *N. thynnoides* (Cuvier, 1829) by Chen et al. (2010) revealed that they were a misidentification of the same new species. The purposes of present work are to name and describe the new species; to morphologically compare it with all nominal species of *Naso*; and to discuss its phylogenetic relationship with other congeners.

MATERIAL AND METHODS

Morphological measurements and counts were taken in the manner following Randall & Bell (1992). Data for comparison is that provided by Randall & Bell (1992), including only specimens between 300–400 mm SL, including the 369 mm holotype of *N. caesius*. All specimens were dissected to examine the gonad for sex determination. Sagittal otoliths were extracted and digestive tracts were dissected from five specimens for further studies. Otolith measurements and character descriptions follow Smale et al. (1995). Abbreviations for institutions follow Fricke and Eschmeyer (2011, online version).

The same three genetic markers (ETS2, 16S, and Cyt *b*) as per Klanten et al. (2004) were used to obtain a detailed resolution of the new species within the genus *Naso*. The nuclear intron ETS2 (Lyons et al., 1997) and two mt markers, 16S rRNA (Simon et al., 1994) and cytochrome *b* (Kocher et al., 1989) were PCR-amplified for four specimens. PCR was modified from Klanten et al. (2004) and performed in a Biometra TGradient Thermocycler with a 15- μ L reaction volume contained 0.2 μ M dNTPs, 1.5 μ L of 10 \times PCR buffer (Bioman, Taipei, Taiwan), 0.5 μ M each of forward and reverse primer, 0.2 U Taq DNA polymerase (Bioman, Taipei, Taiwan), and 10 ng of template DNA. DNA amplification for the three target genes were carried out using the following PCR conditions: 35 cycles of denaturation at 94°C for 15, annealed at 55°C for ETS2, 52°C for Cyt *b* and 54°C for 16S, respectively for 15 and extension at 72°C for 30. A final extension was at 72°C for 10 min. Although PCR products of ETS2 had two bands (around 500 and 150 bp sizes), the sequencing from both directions can exclude the noise sequencing fragments and get a full ETS fragment (483 bp) after combining the sequencing results from both directions without the need for further PCR product purification. The templates of 16S and Cyt *b* were also sequenced directly as described above. Sequences were analysed on an automated ABI Prism 377 sequencer at Taiwan Normal University Sequencing Facility.

All genes were combined and automatically aligned using MAFFT version 6 (Kato et al., 2002). Phylogenetic trees were reconstructed using the maximum likelihood (ML) methods and Bayesian analysis. By using the Akaike information criterion (AIC) in MEGA5, the transversion model with invariable sites GTR+G+I (Gamma = 0.55, Ts/Tv ratio = 5.22) was selected for construction of the ML tree with *Prionurus microlepidotus* as an out-group. Bayesian analysis was performed in MrBayes 3.0 (Huelsenbeck & Ronquist, 2001) using Markov chain Monte Carlo (MCMC) search with 4 chains of 1 million generations. Trees were sampled every 100 generations, and the first 250,000 generations were discarded as burn-in. Nodes with bootstrap values \geq 70% were considered well supported (Hillis & Bull, 1993). All sequences obtained in this study were deposited in GenBank.

TAXONOMY

Naso tergus, new species

(Figs. 1–4; Table 1)

Naso thymoides (non-Cuvier, 1829): Chen et al., 2010:542 (in part, the figured specimen is misidentification of present species).

Material examined. – Holotype: NMMB-P10808 (adult male, 335 mm SL), off Nanfangao, Ilan, NE Taiwan, NW Pacific Ocean, 70–80 m, bottom gill net, 15 Dec.2010.

Paratypes: NMMB-P10809 (1, adult female, 325); NMMB-P10810 (1, fully ripe female, 320); NMMB-P10811 (1, adult male, 347); NMMB-P10812 (1, adult female, 321); NMMB-P10813 (1, adult female, 320), otolith and gut taken; NMMB-P10814 (1, adult female,

303), otolith and gut taken; NMMB-P10815 (1, adult female, 306), otolith and gut taken; NMMB-P10816 (1, adult male, 342), otolith and gut taken; NMMB-P10817 (1, adult female, 302), otolith and gut taken; QM I.38837 (1, adult male, 315); all collected together with the holotype. NMMB-P2373 (1, adult male, 280), Houbihu fish market, Pingtung, S. Taiwan, N. South China Sea, 6 Feb.2002. NMMB-P10480 (1, adult female, 275), Houbihu fish market, Pingtung, S. Taiwan, N. South China Sea, 1 Dec.2002.

Diagnosis. – A species of *Naso* differs from congeners in having VI dorsal-fin spines; an acute head, without rostral projection or tuberosity in adults; two bony plates of caudal peduncle; a relatively large eye (6.3–7.2% SL); a median body depth, 2.8–2.9 in SL; 100–120 teeth on upper jaw and 90–110 on lower jaw; a uniformly brownish body coloration; a relatively small adult size which might not exceed 350 mm SL; and a different genome structure.

Description. – The following proportions are given for the holotype, followed by range of all types in parentheses, if different from the holotype. Dorsal-fin rays VI, 28 (VI, 26–30; mainly VI, 28); anal-fin rays II, 27 (II, 26–28; mainly II, 27); pectoral-fin rays 16 (1 unbranched+14 branched and 2 unbranched); pelvic-fin rays I, 5; gill rakers 4+13=17 (4+11–13=15–17). Head length 3.9 (3.8–4.1) in SL; body depth 2.9 (2.7–2.9); body width 6.8 (6.6–7.5); predorsal length 3.6 (3.5–4.0); prepectoral length 4.0 (3.6–4.3); prepelvic length 3.4 (3.1–3.4); preanal length 2.6 (2.4–2.6). Snout length 1.9 (1.8–1.9) in HL; orbit diameter 2.7 (2.6–2.8); caudal peduncle length 3.0 (2.6–3.1); caudal peduncle depth 4.8 (4.8–5.6); caudal peduncle width 3.8 (3.8–4.6); upper jaw length 4.8 (4.5–5.2); first dorsal-fin spine length 2.7 (2.0–4.7); sixth dorsal-fin spine length 2.9 (2.7–3.0); pectoral-fin length 1.8 (1.7–1.8); pelvic-fin spine length 2.9 (2.4–3.9); first anal-fin spine 4.3 (3.3–6.8); 2nd anal-fin spine 3.6 (3.2–4.5); caudal-fin length 1.0 (0.9–1.1).

Two elliptical bony plates on each side of caudal peduncle, the anterior plate usually larger than the posterior one and about equal size in adult males; a keel-like spine projecting laterally in adults, more in males than females; shape of peduncular spines semi-circular and slightly pointed with the tip projecting forward in adult males (Fig. 2).

Body moderately elongated and compressed, the lateral profile spindle-like, deepest at midpoint of body; head relatively large, dorsal profile gradually sloping with a small convexity usually present at about level of eye; interorbital arched well above eyes; mouth relatively small, upper jaw slightly projecting; gape horizontal to slightly dorsally inclined; teeth uniserial, slender, lanceolate, tips slightly curved posteriorly, 100–120 in upper jaw, 90–110 in lower jaw (usually 10 more in upper than lower), the numbers do not vary relative to size among the type series; lips narrow; tongue broadly rounded.

A deep oblique groove on snout from in front of middle of eye to nearly two-thirds distance to front of mouth; nostrils very small, just above deep groove and anterior to middle of eye by a distance about one-third orbital diameter; anterior nostril with a low membranous rim and a small triangular

Table 1. Morphometric data of type series of *Naso tergus*, new species and non-type specimens of *N. hexacanthus*.

	<i>Naso tergus</i> , new species			<i>Naso hexacanthus</i>
	Holotype	All types	SD	Non-type
Standard length (SL, in mm)	335	275–335 (n=13)		295–305 (n=2)
% SL		Mean (Range)	SD	Range
Head length	25.5	25.5 (24.6–26.6)	0.6	25.1–25.4
Body depth	34.5	35.1 (34.4–36.8)	0.7	39.1–39.6
Body width	14.8	14.5 (13.3–15.2)	0.5	12.4–13.9
Predorsal length	27.5	27.3 (25.2–28.7)	0.9	26.9–27.0
Prepectoral length	24.9	25.3 (23.5–27.6)	1.1	23.4–23.6
Prepelvic length	29.3	30.3 (29.3–32.2)	0.9	27.5–28.7
Preanal length	38.6	39.7 (38.6–41.3)	0.9	35.9–36.1
Snout length	13.8	13.6 (13.2–14.1)	0.3	14.3–14.8
Eye diameter	6.5	6.7 (6.3–7.2)	0.3	5.3–5.4
Interorbital width	9.5	9.5 (8.9–10.0)	0.3	8.6–8.7
Upper jaw length	5.3	5.3 (4.9–5.7)	0.2	5.0–5.1
Suborbital width	8.8	9.0 (8.6–10.0)	0.4	9.7–10.3
1st dorsal-fin spine length	9.4	9.6 (5.5–12.7)	2.0	9.8–10.3
2nd dorsal-fin spine length	–	9.3 (8.6–10.8)	0.7	9.8–11.1
3rd dorsal-fin spine length	9.0	9.0 (8.2–10.7)	0.7	11.0–11.1
4th dorsal-fin spine length	8.1	9.2 (8.1–10.3)	0.6	10.0–10.5
5th dorsal-fin spine length	9.9	9.6 (8.8–11.7)	0.8	10.3–11.1
6th dorsal-fin spine length	8.9	9.1 (8.4–9.7)	0.5	10.3–11.2
Pectoral fin length	14.4	14.9 (14.0–15.7)	0.6	15.2–16.4
Pelvic-fin spine length	8.9	9.4 (8.0–10.5)	0.8	10.0–10.9
1st anal-fin spine length	6.0	6.2 (3.6–7.9)	1.3	6.1–6.9
2nd anal-fin spine length	7.0	7.0 (5.6–8.6)	0.9	5.9–7.0
Caudal peduncle length	8.4	8.8 (7.8–9.9)	0.6	8.0–8.2
Caudal peduncle depth	5.3	4.8 (4.3–5.3)	0.3	4.5–4.6
Caudal peduncle width	6.7	6.1 (5.4–6.7)	0.5	5.2–5.2
Caudal fork length	16.7	16.9 (16.2–18.0)	0.6	18.0–18.0
Caudal fin length	25.6	25.2 (23.0–28.1)	1.4	27.9–28.0



Fig. 1. *Naso tergus*, new species: a, Holotype, NMMB-P10808, adult male, 335 mm SL; b, Paratype, NMMB-P10813, adult female, 320 mm SL.



Fig. 2. Ventral view of caudal peduncle of *Naso tergus* shows the keel-like spines on peduncular plates, anterior to right. From the holotype.

posterior flap which barely extends posteriorly; posterior nostril rounded in shape.

Scales very small, close-set, the margins not apparent without magnification, each with an elevated dense patch of posteriorly directed spinules (thus the texture is smooth when stroked posteriorly, but finely abrasive in the forward direction); body and head completely scaled except for lips, opercular membrane, and a narrow zone around anterior nostril; membranes of fins scaleless; spines, and to a lesser extent soft rays, scaled laterally (pelvic and first dorsal and anal spines scaled anteriorly as well). Lateral line on upper side of body approximately following contour of back; no slit-like pores above lateral line (as in *N. caesius*) can be observed.

Origin of dorsal fin well posterior to a vertical at upper end of gill opening, as well as posterior margin of operculum; dorsal and anal spines slender but transversally broad basally, especially the first in each fin; all dorsal spines about equal in length; first dorsal-fin ray slightly longer than sixth dorsal-fin spine; first two rays unbranched; successive rays progressively slightly longer to about fourteenth ray; the last ray shortest and forked to base. Origin of anal fin at a vertical from base of fourth dorsal-fin spine; first anal-fin spine usually shorter than the second spine; lengths of anal-fin rays almost similar to those of dorsal fin; caudal fin emarginated (apparently not truncate even in adults); origin of pectoral fin at a vertical of posterior margin from operculum; pectoral fin short, first two and last rays unbranched, the third to fifth rays longest; origin of pelvic fin opposite that of dorsal fin, the appressed fin not reaching the origin of anal fin; pelvic fin short, the first soft ray longest, about equal to the spine in length. Anus situated above second third of appressed pelvic fins.

Coloration. – When fresh, uniformly light brownish or greenish above and pale below. When preserved, uniformly light gray-brownish, paler ventrally, with no obvious dark or light markings on head or body; caudal fin uniformly brown; pectoral fin light brown, the membranes pale distally; membranes of dorsal and anal fins blackish, without any markings; pelvic fin pale; tongue and gill rakers creamy white.

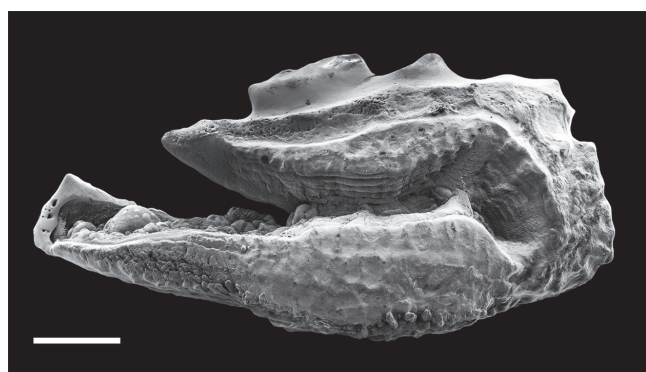


Fig. 3. Scanning microscopy image of the mesial face of right sagittal otolith taken from *Naso tergus*, NMMB-P10816, adult male, 342 mm SL. Scale bar = 1 mm.

Distribution. – Known from the type series, collected from off northeastern and southern Taiwan by bottom gill-net and hook and line at depths between 70 and 80 meters.

Size. – The mean body size of males is slightly larger than that of females. All four males have large, white, band-like testes and the largest specimen is 347 mm SL. All females have large ovaries with eggs in or approaching the final stage, the largest specimen is 325 mm SL.

Otolith. – Sagittal otolith oblong-ovate with moderate thickness, convex in mesial and slightly concave in lateral faces. Dorsal margin, slightly raised with irregular sculpture; ventral margin, slightly rounded and crenate; posterior margin, flattened to rounded and irregular. Rostrum, elongate, moderately broad and sharply rounded; antirostrum, small and pointed; excisura, wide, notch very deep or shallow with groove, angle acute. Sulcus acusticus, ostial and heterosulcoid; ostium, very elongate and narrow, lying mainly on rostrum, deepest in middle; cauda, slightly sinuate, strongly flexed, very deep posteriorly undercutting ventral area; ostium/cauda ratio, 1/0.60–0.64 (Fig. 3).

Etymology. – The species name, *tergus*, meaning ‘to hide’, refers to the typical appearance of this species that makes it resemble subadults of many other *Naso* species.

Feeding habit. – Benthic crustaceans and bivalves in the stomach and intestine of *N. tergus* indicate that this fish feeds on marine organisms living on the sea floor.

Comparison. – *Naso tergus* is morphologically similar to *N. caesius* but differs in having a relatively short caudal peduncle (7.8–9.9%, vs. 13.6–14.2% SL in *N. caesius*); a relatively large eye (6.3–7.2% vs. 5.0–5.6% SL); a relatively wide interorbital distance (8.9–10.0% vs. 7.9–8.6% SL); caudal fin emarginated in adults (vs. truncate); a relatively large head (24.6–26.6% vs. 23.8–24.3% SL); a relatively shallow body depth (34.4–36.8% vs. 36.2–39.0% SL); a relatively wide body (13.3–15.2% vs. 12.1–13.2% SL); a relatively long prepelvic distance (29.3–32.2% vs. 26.9–27.7% SL); a relatively long preanal distance (38.6–41.3% vs. 34.7–35.9% SL); modally 16 pectoral-fin rays (vs. 17); and a relatively small adult body size (up to 347 mm SL, vs. up to 625 mm SL in Randall, 2002). Randall & Bell (1992) mentioned there was a curious series of about 20 vertical slit-like pores in a row a few mm above and parallel to the lateral line, however, it is not observed in *N. tergus*.

Naso tergus is also similar to *N. hexacanthus* (data of 2 specimens is provided in Table 1) but differs in having a uniformly light brown colour (vs. irregular lines on dorsal and anal fins, yellowish ventrally, black borders on opercle and subopercle and a white lower lip in *N. hexacanthus*); modally 16 pectoral fin rays (vs. 17); a relatively large eye (6.3–7.2% SL vs. 5.3–5.4% SL); modally 12 gill rakers on lower limb of first gill arch (vs. 8–10); body depth 2.8–2.9 in SL (vs. 2.5–2.6); relatively small prepectoral, prepelvic and preanal length; relatively long dorsal-fin spines; and a pale tongue and gill rakers (vs. both blackish).

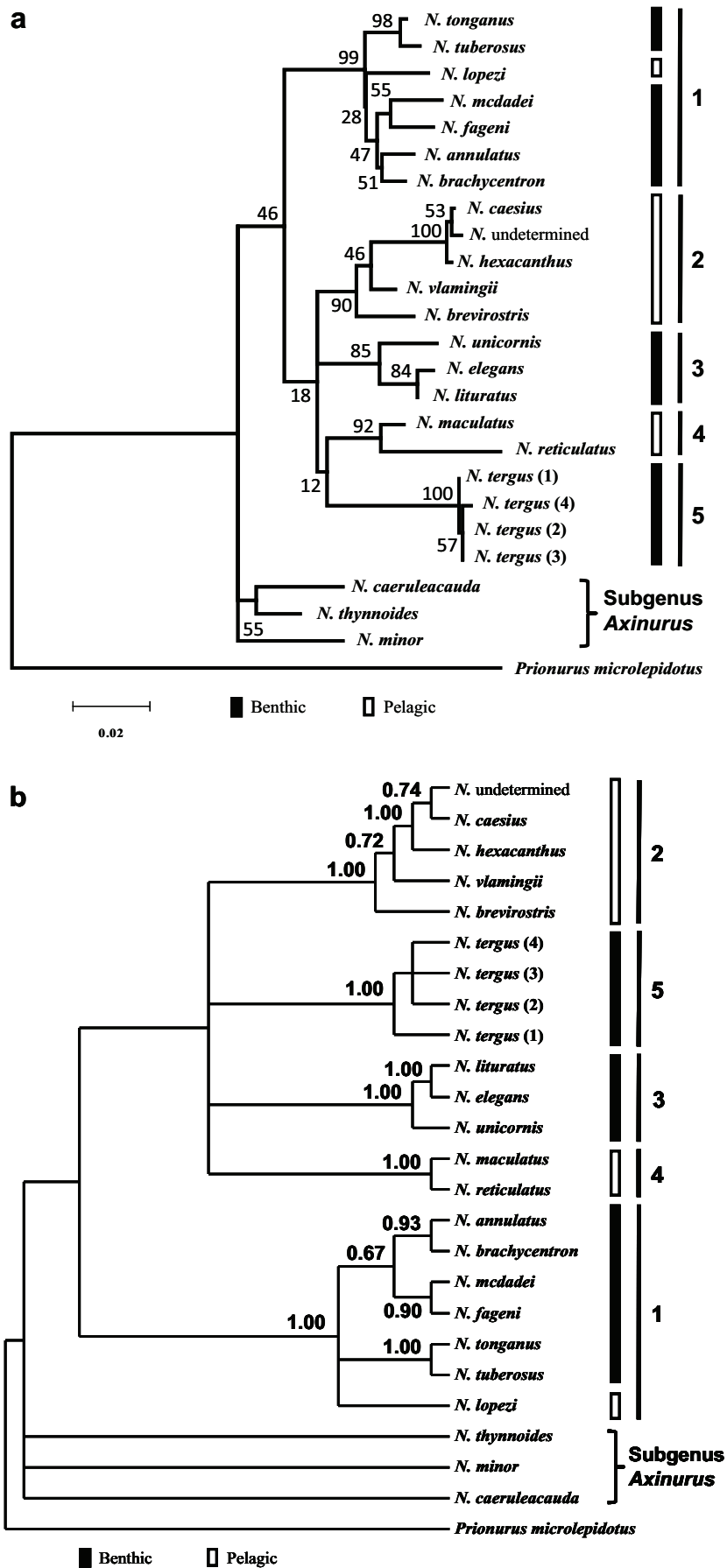


Fig. 4. Phylogenetic trees of *Naso* species using combined DNA data (ETS2, 16S and Cyt *b*): a, Topology by ML analysis and bootstrap values at the branches; b, Topology by Bayesian analyses and posterior probabilities at the branches. Numbers refer to the sub-clades: 1 for the *N. annulatus* sub-clade, 2 for the *N. brevisrostris* sub-clade, 3 for the *N. elegans* sub-clade, 4 for the *N. maculatus* sub-clade, and 5 for the *N. tergus* sub-clade. Black and open squares indicate the foraging modes.

Moreover, the presence of two caudal plates separates *N. tergus* from *N. caeruleacauda* Randall, 1994, *N. minor* (Smith, 1966) and *N. thynnoides*, which have only one plate; the absence of a rostral protuberance in adults separates it from *N. annulatus* (Quoy & Gaimard, 1825), *N. brachycentron* (Valenciennes in Cuvier & Valenciennes, 1835), *N. brevirostris* (Valenciennes in Cuvier & Valenciennes, 1835) and *N. unicornis* (Forsskål, 1775); the absence of markings or a distinctive colour pattern on body or fins separates it from *N. brevirostris*, *N. elegans* (Rüppell, 1829), *N. lituratus* (Forster, 1801), *N.lopezi* Herre, 1927, *N. maculatus* Randall & Struhsaker, 1981, *N. reticulatus* Randall, 2001 and *N. vlamingii* (Valenciennes in Cuvier & Valenciennes, 1835); the relatively shallow body separates it from *N. brachycentron*, *N. elegans*, *N. lituratus*, *N. tuberosus* Lacepède, 1801, *N. unicornis* and *N. vlamingii*; the absence of a hump on the back separates it from *N. brachycentron*, *N. tonganus* (Valenciennes in Cuvier & Valenciennes, 1835) and *N. tuberosus*; the absence of filamentous caudal fin rays separates it from *N. elegans*, *N. fageni* Morrow 1954, *N. unicornis*, and *N. vlamingii*; the absence of a tuberosity on the snout separates it from *N. fageni*, *N. mcdadei* Johnson, 2002, *N. tonganus* and *N. tuberosus*; and the relatively small adult body size separates it from most congeners, except for *N. minor*, *N. thynnoides* and *N. brevirostris*. Our new species is also different from two possible undescribed species mentioned in Chen et al. (2010) in lacking black spots on the sides of the body.

Phylogenetic reconstruction. – Three DNA genes of four *N. tergus* specimens were analysed and compared with the molecular data of same sequences provided by Klanten et al. (2004). The new species is well separated from other species of *Naso* by the molecular data (Fig. 4). According to the maximum likelihood phylogenetic tree, *N. tergus* is in the *N. maculatus* sub-clade (sensu Klanten et al., 2004: 228) but with a very low bootstrap support. Maximum likelihood phylogenetic tree also has low bootstrap support among *N. brevirostris*, *N. elegans* and *N. maculatus* sub-clades and even between *N. annulatus* and other sub-clades (Fig. 4a). Bayesian posterior probabilities, however, have better support for all major sub-clades and suggest the new species could be regarded as the fifth sub-clade for *Naso* genus based on the same clade classification criteria (Klanten et al., 2004).

Two issues arise from the phylogenetic reconstruction reported in this study. Firstly the low bootstrap support for many of the clades suggests the history of this genus was characterized by episodes of rapid evolutionary divergence. Secondly distinct morphologies associated with foraging and feeding can arise independently in each of the clades. This is confirmed by the similarity between *N. caesi*us/*N. hexacanthus* and *N. tergus*, all of which share the streamlined scombiform morphology but occur in distinct clades.

A number of distinctive morphological and structural features arise independently in each of the main clades. Our result on the species relationships within *Naso* differs from that proposed by Borden (1998) on the basis of morphology and demonstrates a conflict by using molecular and morphological characters for the identification of evolutionary groupings in this genus.

ACKNOWLEDGEMENTS

We thank S.-P. Lee (Nanfangao fishing port) for collection of specimens, J. H. Choat (James Cook University) and S. O. Klanten (University of Sydney, Australia) for reviewing the early draft, J. Johnson (QM) for the useful comments and S.-Y. Wang (NMMBP) for curatorial assistance. This study was supported by the research grants NMMBA100200352, 100200354 and NSC99-2631-H-291-001.

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